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**Tumor-associated trypsin inhibitor (TATI) and tumor-associated
trypsin-2 (TAT-2) predict outcomes in gastric cancer**

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Abstract

Introduction: Tumor-associated trypsin inhibitor (TATI) limits serine proteases, promotes carcinogenesis in several cancers and functions as an acute-phase reactant. Tumor-associated trypsin-2 (TAT -2), a proteolytic target enzyme for TATI, can enhance invasion by promoting extracellular matrix degradation. Here, we aimed to study serum TATI and TAT-2 levels, including the TAT-2/TATI ratio, as prognostic and diagnostic biomarkers in gastric cancer. We compared the results with the plasma level of C-reactive protein (CRP).

Materials and Methods: We selected 240 individuals operated on for gastric adenocarcinoma at the Helsinki University Hospital, Finland, between 2000 and 2009. We determined the preoperative serum TAT-2, TATI and plasma CRP levels using time-resolved immunofluorometric assays using monoclonal antibodies.

Results: The medium serum TAT-2 level was higher among gastric cancer patients [8.68 ng/ml; interquartile range (IQR) 5.93–13.2] than among benign controls (median 5.41 ng/ml; IQR 4.12–11.8; $P = 0.005$). Five-year survival among patients with a high serum TAT-2 was 22.9% [95% confidence interval (CI) 11.7–34.1], compared to 52.2% (95% CI 44.6–59.8; $p < 0.001$) among those with a low level. The five-year survival among patients with a high serum TATI was 30.6% (95% CI 20.4–40.8), compared to 52.9% (95% CI 44.7–61.1; $P < 0.001$) among those with a low level. The serum TATI level remained significant in the multivariable survival analysis (hazard ratio 2.01; 95% CI 1.32–3.07). An elevated plasma CRP level associated with a high serum TATI level ($P = 0.037$).

Conclusion: This study shows for the first time that a high serum TAT-2 may function as a prognostic biomarker in gastric cancer and that TAT-2 levels may be elevated compared to controls. Additionally, we show that the prognosis is worse among gastric cancer patients with a high serum TATI. These biomarkers serve as prognostic factors particularly among patients with a metastatic or a locally advanced disease.

Key words: Gastric cancer, biomarkers, tumor-associated trypsin inhibitor, tumor-associated trypsin-2, prognosis

Introduction

Gastric adenocarcinoma represents one of the most lethal cancers worldwide [1]. In the early stages of the disease, symptoms are scarce; when severe symptoms appear, however, the disease has often already spread. None of the tumor markers currently clinically available have demonstrated a sufficient sensitivity to be useful for detecting gastric cancer and carry a limited prognostic value [2].

Tumor-associated trypsin inhibitor (TATI), also known as the pancreatic secretory trypsin inhibitor (PSTI), is a serine protease inhibitor first found in the urine of a patient with gynecological cancer [3,4], called serine peptidase inhibitor Kazal-type 1 (SPINK1). In addition to inhibiting serine proteases, it appears to promote carcinogenesis by activating the epidermal growth factor receptors (EGFRs) [5,6]. In addition to gastric cancer, TATI expresses in several other cancers, including colorectal, pancreatic, ovarian, bladder, renal, prostate, lung, breast and liver cancers, and appears useful for both diagnostic and prognostic purposes [6]. The prognostic role of TATI in gastric cancer remains controversial since, on the one hand, a high TATI expression in gastric carcinoma tissues served as a marker of a favorable outcome and associated with a low cancer stage, superficial tumors and the absence of metastasis. On the other hand, higher serum TATI levels were found more often among gastric cancer patients than among controls and associated with advanced disease [7,8]. Additionally, TATI acts as an acute-phase reactant and the regulation of its synthesis resembles that of the C-reactive protein (CRP) and other acute-phase reactants [9,10]. Thus, in addition to malignant diseases, an elevated serum TATI level may result from an inflammation caused by an infection or tissue damage [11,12].

Tumor-associated trypsin-2 (TAT-2) represents one of the proteolytic target enzymes for TATI [13]. A high TAT-2 expression enhances a tumor's invasive capacity

84 through increased extracellular matrix degradation via the activation of pro-urokinase
85 and matrix metalloproteinases [14,15]. Moreover, a surplus of TAT-2 compared to
86 TATI, leading to a high TAT-2/TATI ratio, may result in increased TAT-2 activity [6].
87 Furthermore, TAT-2 expresses in several cancers, including gastric cancer, yet the
88 background levels of pancreatic secretory trypsin limit its clinical use in diagnosing
89 pancreatitis [16–19]. In a previous study of serum trypsinogen in gastric cancer patients,
90 TAT-2 levels were significantly higher among those with a linitis plastica–type gastric
91 cancer compared to those with a non-linitis plastica–type gastric cancer [20]. Likewise,
92 trypsin production by gastric cancer cells associates with an increased in vitro
93 invasiveness of the tumor cells [17,21]. Serum TAT-2 has not been previously studied
94 in gastric cancer as a prognostic biomarker.

95 CRP has been studied in several cancers in attempts to clarify the mechanism
96 behind the systemic inflammatory reaction sometimes seen in cancer [22]. In colon
97 cancer, after excluding patients undergoing emergency surgery and those with auto-
98 immune disease or infection, an elevated preoperative CRP predicts a poor outcome
99 [23]. A high preoperative CRP was also shown to indicate a poor outcome following
100 surgical resection of pancreatic ductal adenocarcinoma or colorectal liver metastases
101 [24,25]. Among gastric cancer patients, an elevated preoperative CRP served as a
102 marker of a poor outcome and associated with metastatic disease [26,27]. Similarly, a
103 recent meta-analysis consisting of 2597 gastric cancer patients concluded that an
104 elevated preoperative CRP predicts a poor outcome [28].

105 Here, we aimed to study serum TATI and TAT-2 levels, including the TAT-
106 2/TATI ratio, as prognostic and diagnostic biomarkers in gastric cancer. We also
107 compared these with gastric cancer patients' plasma CRP levels.

Materials and Methods

Patients and ethics

Our cohort consisted of 240 patients operated on for gastric adenocarcinoma in the Department of Surgery, Helsinki University Hospital, Finland, between 2000 and 2009, as previously described [29]. Patients undergoing surgery were consecutively included in this study, excluding individuals with a history of malignant disease or synchronous cancer. Each gastric tumor was histologically verified as gastric adenocarcinoma by an experienced pathologist from the Department of Pathology. We used the seventh version of the Tumor-Node-Metastasis (TNM) classification for the cancer staging [30]. We compared gastric cancer patients to 48 control patients undergoing surgery or gastroscopy to treat and diagnose a variety of non-malignant conditions between 2000 and 2012 (Supplementary Table 1).

We updated survival data in August 2019. Data were received from patient records, the Population Register Center of Finland and Statistics Finland.

The Surgical Ethics Committee of Helsinki University Hospital (Dnro HUS 226/E6/ 06, extension TMK02 §66 17.4.2013) approved the study protocol. We were authorized to study the archived tissue samples retrospectively without requiring further individual consent or permission as issued by the National Supervisory Authority of Welfare and Health (Valvira Dnro 10041/06.01.03.01/2012).

Serum and plasma samples

Blood samples were drawn 1 to 30 days (median, 1 day) before surgery. Serum and plasma were separated, aliquoted and stored at -80°C until analysis, according to the routine procedures of the hospital laboratory.

To determine the gastric cancer patients' plasma CRP levels, we applied a high-sensitivity method [time-resolved immunofluorometric assay (TR-IFMA) in microtitration plates using a monoclonal CRP antibody (anti-hCRP, code 6405, Medix Biochemica, Espoo, Finland)], as detailed elsewhere [24]. The gastric cancer patients' serum TATI and TAT-2 levels were also determined using TR-IFMA, which we described previously [31,32].

Statistical analyses

We explored the strength of associations between biomarker levels and clinicopathological variables among gastric cancer patients using the Mann-Whitney U-test and the Kruskal-Wallis test. Correlations were evaluated using the Spearman's rank correlation. We dichotomized the variables for the survival analyses. The cut-off value for CRP was 10 mg/l. In the survival analyses, cut-off values for TAT-2, TATI, and the TAT-2/TATI ratio were determined using the maximum value for Youden's index to find the best prognostic performance among our cohort of gastric cancer patients [33]. Furthermore, validation analyses were conducted using median cut-off values for TAT-2 and TATI (Supplementary Table 4). We determined the significant difference in biomarker levels between gastric cancer patients and controls using the Mann-Whitney U-test. Additionally, in studying the diagnostic value of TAT-2, the receiver operating characteristic (ROC) curve analysis was applied to study the area under the curve and to determine a distinct cut-off value for TAT-2 to calculate the sensitivity, specificity, predictive values, and accuracy. The cut-off value was determined by the maximum value of Youden's index to optimally differentiate gastric cancer patients from controls, with equal values for sensitivity and specificity. The Kaplan-Meier method was applied to create survival curves, and the statistical significance was calculated using the log-

rank test. We used the Cox proportional hazard model to calculate the hazard ratios (HRs) for the uni- and multivariable survival analyses. Covariates entered into the multivariable survival analysis consisted of age, stage, the Laurén classification and the serum TAT-2 and TATI levels. Cancer stage was processed as a categorical covariate, in which we compare patients with higher cancer stages to those with stage I cancer. Furthermore, we studied gastric cancer patients' survival in several subgroups using the Cox proportional hazard model and visually depicted it using the Kaplan-Meier estimators. The patient subgroups analyzed were stratified by age, gender, TNM-classification, stage, the Laurén classification, and the CRP, TAT-2, and TATI levels. The primary outcome in all of the survival analyses was death due to gastric cancer. We calculated the disease-specific survival from the date of surgery to the end of follow-up or death due to gastric cancer. Individuals leaving the study before the end of follow-up and individuals dying of causes other than gastric cancer were censored. We considered a two-tailed P-value of less than 0.05 as statistically significant across all analyses. We used IBM's SPSS Statistics for Mac version 24.0 (IBM Corporation, Armonk, NY, USA) for all analyses.

Results

Diagnostic value of TAT-2, TATI, TAT-2/TATI ratio and CRP

Serum TAT-2 levels were higher among gastric cancer patients [median 8.68 ng/ml; interquartile range (IQR) 5.93–13.2] than controls (median 5.41 ng/ml; IQR 4.12–11.8; $P = 0.005$; Supplementary Table 2). The area under the ROC curve was 0.63 (95% CI 0.53–0.72; $P = 0.005$). The maximum value of Youden's index was used to determine

the optimal cut-off value (5.45 ng/ml) for TAT-2 to calculate the sensitivity, specificity, predictive values and accuracy among gastric cancer patients and controls. The sensitivity of TAT-2 to distinguish gastric cancer patients from controls reached 78.3% with a specificity of 52.1%. The positive predictive value reached 89.1%, with a negative predictive value of 32.5% and an accuracy of 74.0%. The prevalence of gastric cancer in this test population reached 83.3%. We found no significant differences in the TATI or CRP levels or in the TAT-2/TATI ratio between gastric cancer patients and controls.

Associations between TAT-2, TATI, TAT-2/TATI ratio, CRP and clinicopathological variables

We found statistically significant associations between a high serum TAT-2 level and pT3–4 tumors ($P = 0.046$) and between high serum TAT-2 and TATI levels ($P < 0.001$; Table 1). We also identified statistically significant associations between a high serum TATI and an older age ($P = 0.001$), between a high serum TATI and pT3–4 tumors ($P = 0.030$) and between a high serum TATI and an elevated CRP ($P = 0.037$). Furthermore, we discovered statistically significant associations between a high serum TAT-2/TATI ratio and diffuse-type tumors ($P = 0.004$) and between a high serum TAT-2/TATI ratio and a younger age ($P < 0.001$; Supplementary Table 3). We also identified a positive correlation between the TAT-2 and TATI levels ($r_s 0.438$; $P < 0.001$).

Plasma CRP was elevated among older patients ($P = 0.024$), patients with pT3–4 tumors ($P = 0.047$) and among patients with intestinal-type tumors ($P = 0.006$; Supplementary Table 3). We found no other statistically significant associations

between the studied biomarkers and the clinicopathological variables. [Table 1 near here]

Survival analyses

Optimal cut-off values for the survival analyses for TAT-2, TATI and the TAT-2/TATI ratio were determined using the maximum value for Youden's index (for TAT-2, 12.91 ng/ml; for TATI, 18.76 ng/ml; and for the TAT-2/TATI ratio, 0.58). Five-year disease-specific survival among patients with a high serum TAT-2 was 22.9% [95% confidence interval (CI) 11.7–34.1], and 52.2% among patients with a low TAT-2 (95% CI 44.6–59.8; $P < 0.001$; Table 2; Figure 1A). The five-year survival among patients with a high serum TATI was 30.6% (95% CI 20.4–40.8), and 52.9% among patients with a low TATI (95% CI 44.7–61.1; $P < 0.001$; Figure 1B). The serum TAT-2/TATI ratio or plasma CRP did not function as significant prognostic factors in the whole cohort.

Serum TATI remained an independent prognostic factor in the multivariable survival analysis [[hazard ratio \(HR\) 2.01; 95% CI 1.32–3.07; Table 2](#)]. Other significant prognostic factors in the multivariable survival analysis included age, stage and histological type according to the Laurén classification. No significant interaction terms were identified in the final model. [Table 2 and Figure 1 near here]

Survival analyses by subgroups

Among patients with pT3–4 tumors, a high TAT-2 level (Figure 1C) and a high TATI level (Table 3; Figure 1D) served as markers of a poor outcome. A high TAT-2 level served as a marker for an adverse outcome among those with a low TATI level (Figure 1E). Similarly, a high TATI level served as a marker for a poor outcome among those with a low TAT-2 level (Figure 1F).

Furthermore, a high TAT-2 level served as a marker for a poor outcome among those with lymph-node metastasis and among those without lymph-node metastasis (Table 3; Figure 2A). Correspondingly, a high TATI level served as a marker for a poor outcome among those with lymph-node metastasis and among those without lymph-node metastasis (Figure 2B). A high TAT-2 level emerged as indicative of a poor outcome among those with either intestinal- or diffuse-type tumors (Figure 2C). Likewise, a high TATI level served as a marker for a poor outcome among those with either intestinal- or diffuse-type tumors (Figure 2D). A high TAT-2 level served as a marker for a poor outcome among those with an elevated CRP (Figure 2E) and among those without an elevated CRP. Similarly, a high TATI level served as a marker for a poor outcome among those with an elevated CRP (Figure 2F) and among those without an elevated CRP. Additionally, TAT-2 and TATI served as prognostic biomarkers in several other subgroups presented in Table 3.

A high TAT-2/TATI ratio served as a marker for a poor outcome among older patients (HR 2.26; 95% CI 1.39–3.67). [Table 3 and Figure 2 near here]

Discussion

Here, we show that, in our cohort of 240 gastric adenocarcinoma patients, high serum TAT-2 and TATI levels function as markers for a poor outcome. Both parameters are promising prognostic biomarkers, although only TATI remained significant in the multivariable survival analysis, suggesting its prognostic value is superior to that of TAT-2 in this patient cohort. Nevertheless, to our knowledge, this is the first time that serum TAT-2 has been identified as a prognostic biomarker in gastric cancer. Additionally, we show that the expression levels of these biomarkers correlate positively with gastric cancer. Neither the TAT-2/TATI ratio nor CRP functioned as

prognostic biomarkers in this study. Furthermore, we evaluated the biomarkers' diagnostic properties, demonstrating that serum TAT-2 levels were higher among gastric cancer patients than among benign controls.

High serum TAT-2 and TATI levels predicted an unfavorable outcome in several of the subgroups we studied. Interestingly, they did not function as prognostic factors among patients with pT1–2 tumors or with stage I, II or III disease, but among those with pT3–4 tumors or stage IV disease. Because high TAT-2 and TATI levels were also associated with pT3–4 tumors, the mechanisms via which a high level of TAT-2 or TATI affect disease progression appear important in locally advanced or metastatic disease rather than in early-stage gastric cancer. Specifically, TAT-2 enhances the tumor's invasive abilities via the increased degradation of the extracellular matrix through the activation of pro-urokinase and matrix metalloproteinases. TATI, furthermore, promotes tumor growth via the activation of EGFRs. In a clinical setting, our results agree with previous studies [6,14,15]. However, because TAT-2 and TATI also functioned as prognostic factors among patients without lymph-node or distant metastases, their roles in gastric cancer carcinogenesis do not appear limited to the pathogenesis of advanced disease.

This study further clarifies the role of TATI in gastric cancer, as previously described, as associating, on the one hand, with advanced disease and, on the other hand, associating with a favorable outcome and the absence of metastasis [7,8]. A high tissue expression of TATI was previously found to associate with a favorable prognosis, superficial tumors and the absence of lymph-node and distant metastases [7]. Yet, in this study, we found a high serum TATI associated with an adverse prognosis and with locally advanced disease. It, thus, seems that in gastric cancer the expression of TATI may affect disease progression differently depending upon whether it circulates in the

plasma or is expressed in the tumor tissue. TATI was previously studied in the sera of gastric cancer patients yielding results similar to ours, whereby a high serum level associated with locally advanced disease [8]. In that study, survival was not analyzed, although a high TATI level associated with metastasis, indicative of a poor outcome among patients with high TATI levels. Furthermore, in that study, statistically significant differences in the serum TATI levels between gastric cancer patients and controls were found, which we did not find here. The discrepancy between these results may be explained by the choice of controls. We selected controls with benign disease, whereas the previous study relied on healthy individuals.

The expression of trypsinogen using gastric cancer cells was previously shown to associate with invasive growth [17,21]. Here, we showed in a clinical setting that a high serum TAT-2 associated with pT3–4 gastric tumors and a poor outcome, providing evidence of TAT-2's importance in tumor invasion. Similarly, gastric cancer patients' high serum trypsinogen levels were previously shown to associate with a linitis plastica-type cancer, known for its poor prognosis due to its extremely invasive method of growth [20].

TATI and CRP both function as acute-phase reactants, whereby regulating their synthesis was described as resembling that of each other [9,10]. Similarly, we discovered that among gastric cancer patients an elevated CRP and a high TATI level associate with each other. Interestingly, the hazard ratios among patients with high TATI or TAT-2 levels compared to those with low TATI or TAT-2 levels were higher if CRP was elevated. Thus, in patients with a systemic inflammatory response, the mechanisms via which TAT-2 and TATI may affect patient survival appear amplified.

In this study, CRP did not function as a prognostic biomarker nor did we detect differences in the CRP levels between gastric cancer patients and controls. However,

similar to previous studies, we identified an elevated CRP as associated with locally advanced disease [26,27]. A recent meta-analysis of 2597 gastric cancer patients concluded that a high preoperative CRP level predicts a poor outcome. Yet, in 3 of the 12 original studies included in that meta-analysis, CRP failed to function as a prognostic marker, mirroring findings from our study [28]. To understand why studies of CRP as a prognostic biomarker among similar gastric cancer patient cohorts with similar laboratory and statistical methodologies continue to yield conflicting results, further studies on other large, well-defined patient cohorts and further meta-analysis are necessary. We previously showed that plasma CRP, determined using similar methods, functions as a prognostic factor among pancreatic cancer patients, suggesting that the non-significance in this study does not result from an erroneous analysis [24].

Whether or not these two serum markers could be applied in clinical practice in the future remains difficult to determine definitively. Perhaps patients with pT3–4 tumors and high levels of TAT-2 and TATI could benefit from more dose-intensive perioperative chemotherapy or more frequent follow-up. Furthermore, patients with a limited metastatic disease and low TAT-2 and TATI levels could potentially serve as candidates for curative surgical treatment in addition to chemotherapy. Nevertheless, these two parameters remain candidates for further exploration among patients with gastric cancer.

The strengths of this study include the large patient cohort with a long follow-up period consisting of reliable outcome and survival information. The single-center setting limits the generalizability of our findings, and several individual subgroup analyses were conducted in this study, possibly predisposing the results to familywise errors. Cut-off values calculated using Youden's index provide high segregation performance in this patient cohort and cannot be generalized as such. Our results should be validated

in independent patient cohorts, preferably in a multi-center setting. We chose not to include certain well-known risk factors for gastric cancer, such as tumor subsite, perineural and venous invasion and lymphatic emboli in the analyses, since accessing these data in a retrospective manner could introduce inaccuracies. Furthermore, we lack appropriate and detailed data on the chemotherapy administered.

In conclusion, this study shows for the first time that a high serum TAT-2 may function as a prognostic biomarker in gastric cancer and that TAT-2 levels may be elevated compared to controls. Additionally, we show that the prognosis is worse among gastric cancer patients with a high serum TATI. These biomarkers serve as prognostic factors particularly among patients with metastatic or locally advanced disease.

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449 **Disclosure of Interest**

450 The authors report no conflicts of interest.

Figure legends

Figure 1. Gastric cancer patients' disease-specific survival according to the Kaplan-Meier method. P-values were calculated according to the log-rank test. Low versus high A) TAT-2 and B) TATI across the entire cohort. Low versus high C) TAT-2 and D) TATI among patients with pT3–4 tumors. E) Low versus high TAT-2 among patients with a low TATI and F) low versus high TATI among patients with a low TAT-2.

Figure 2. Gastric cancer patients' disease-specific survival stratified by subgroups according to the Kaplan-Meier method. P-values were calculated according to the log-rank test. Low versus high A) TAT-2 and B) TATI among patients with lymph-node metastasis (red lines) and among patients without lymph-node metastasis (black lines). Low versus high C) TAT-2 and D) TATI among patients with intestinal- (red lines) and diffuse-type (black lines) tumors. Low versus high E) TAT-2 and F) TATI among patients with elevated CRP levels.

Table 1. Association of serum TAT-2 and TATI with the clinicopathologic variables and CRP among 240 gastric cancer patients.

	Serum TAT-2 (ng/ml)			Serum TATI (ng/ml)		
	Median	IQR	P value	Median	IQR	P value
Age, years						
<67	8.95	6.59–12.9	0.174 ¹	14.0	11.0–20.7	0.001¹
≥67	8.10	5.17–14.05		18.2	12.1–27.4	
Gender						
Male	9.21	5.85–15.3	0.171 ¹	16.1	11.3–24.6	0.487 ¹
Female	8.11	5.91–11.3		15.7	10.8–22.2	
Stage						
I	8.39	5.25–11.8	0.585 ²	13.4	10.5–20.1	0.284 ²
II	8.89	6.39–13.1		18.0	11.6–26.5	
III	8.56	5.46–12.9		15.7	11.2–23.9	
IV	9.10	6.60–18.5		16.0	11.3–23.4	
Tumor classification (pT)						
pT1–2	7.76	5.00–11.1	0.046¹	13.8	10.6–20.0	0.030¹
pT3–4	9.07	6.30–14.3		16.3	11.6–24.6	
Lymph node metastasis (pN)						
pN0	8.79	6.31–12.4	0.602 ¹	16.0	10.6–23.5	0.479 ¹
pN1–3	8.56	5.33–13.3		16.0	11.3–23.6	
Distant metastasis (pM)						
pM0	8.59	5.64–12.7	0.175 ¹	15.9	11.2–23.6	0.893 ¹
pM1	9.10	6.60–18.5		16.0	11.3–23.4	
Laurén classification						
Intestinal	7.89	5.30–12.7	0.088 ¹	17.7	11.7–26.5	0.064 ¹
Diffuse	9.17	6.33–14.0		15.3	10.8–22.0	
CRP						
<10	8.76	5.88–13.1	0.523 ¹	15.7	11.1–22.4	0.037¹
≥10	8.59	6.40–18.3		19.2	13.0–30.0	
Serum TAT-2						
Low				14.2	10.6–18.6	<0.001¹
High				24.6	17.7–34.3	
Serum TATI						
Low	7.47	4.97–10.3	<0.001¹			
High	13.2	8.10–21.3				

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, IQR = interquartile range

¹Mann-Whitney U-test, ²Kruskal-Wallis test

Table 2. Uni- and multivariable survival analyses for 240 gastric cancer patients.

Disease-specific survival was analyzed.

	Univariable		Multivariable	
	HR	95% CI	HR	95% CI
Age, years				
<67	1.00		1.00	
≥67	1.44	1.07–1.94	2.32	1.61–3.33
Stage				
I	1.00		1.00	
II	4.97	2.05–12.0	6.10	2.08–17.8
III	14.8	6.46–34.0	23.0	8.32–63.5
IV	41.7	17.7–98.1	87.8	30.1–256
Laurén classification				
Intestinal	1.00		1.00	
Diffuse	1.45	1.06–1.98	2.50	1.67–3.74
CRP				
<10	1.00			
≥10	1.29	0.83–1.98		
Serum TAT-2				
Low	1.00		1.00	
High	1.93	1.35–2.76	1.27	0.83–1.95
Serum TATI				
Low	1.00		1.00	
High	1.84	1.31–2.57	2.01	1.32–3.07
Serum TAT-2/TATI ratio				
Low	1.00			
High	1.27	0.91–1.77		

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, HR = hazard ratio, CI = confidence interval

Table 3. Survival analyses by subgroups, high serum TAT-2 and TATI levels compared to low among 240 gastric cancer patients. Disease-specific survival was analyzed.

	High Serum TAT-2		High Serum TATI	
	HR	95% CI	HR	95% CI
Age, years				
<67	1.66	0.99–2.76	1.63	1.00–2.68
≥67	2.24	1.35–3.70	1.86	1.15–3.02
Gender				
Male	1.87	1.13–3.11	1.80	1.09–2.97
Female	2.12	1.27–3.55	1.87	1.19–2.96
Stage				
I	4.73	0.66–33.6	3.48	0.48–25.1
II	2.24	0.90–5.59	1.68	0.71–3.97
III	1.42	0.85–2.38	2.13	1.33–3.41
IV	2.25	1.15–4.41	2.37	1.12–4.62
Tumor classification (pT)				
pT1–2	0.97	0.22–4.35	1.42	0.44–4.54
pT3–4	1.70	1.17–2.47	1.57	1.10–2.23
Lymph node metastasis (pN)				
pN0	3.28	1.27–8.50	3.56	1.38–9.23
pN1–3	1.61	1.08–2.42	1.81	1.24–2.65
Distant metastasis (pM)				
pM0	1.73	1.12–2.67	1.94	1.31–2.89
pM1	2.25	1.15–4.41	2.37	1.21–4.62
Laurén classification				
Intestinal	2.19	1.05–4.57	2.10	1.10–4.02
Diffuse	1.76	1.17–2.66	1.86	1.25–2.78
CRP				
<10	1.65	1.10–2.47	1.67	1.14–2.44
≥10	3.67	1.64–8.22	2.51	1.08–5.84
Serum TAT-2				
Low			1.80	1.15–2.83
High			1.02	0.52–2.02
Serum TATI				
Low	2.30	1.21–4.36		
High	1.25	0.75–2.08		

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, HR = hazard ratio, CI = confidence interval

Supplementary Table 1. Indications for surgery or gastroscopy among the 48 control patients.

Indication	Count
Peptic ulcer disease	12
Benign gastrointestinal tumor	8
Melena	7
Biliary obstruction	6
Hematemesis	5
Abdominal hernia	4
Adrenal hyperplasia	2
Other non-malignant disease	4

Supplementary Table 2. Serum TAT-2, TATI, TAT-2/TATI ratio, and CRP among gastric cancer patients versus controls.

	Gastric Cancer		Controls		P value ¹
	Median	IQR	Median	IQR	
TAT-2 (ng/ml)	8.68	5.93–13.2	5.41	4.12–11.8	0.005
TATI (ng/ml)	15.9	11.2–23.5	14.5	10.3–20.1	0.357
TAT-2/TATI ratio	0.55	0.36–0.86	0.46	0.26–0.78	0.063
CRP (mg/l)	1.62	0.51–5.81	2.04	0.42–8.44	0.627

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, IQR = interquartile range

¹Mann-Whitney U-test

Supplementary Table 3. Association of CRP and TAT-2/TATI ratio with clinicopathologic variables among 240 gastric cancer patients.

	CRP (mg/l)			Serum TAT-2/TATI ratio		
	Median	IQR	P value	Median	IQR	P value
Age, years						
<67	1.12	0.48–3.49	0.024¹	0.64	0.41–1.04	<0.001¹
≥67	2.19	0.56–7.80		0.45	0.33–0.67	
Gender						
Male	1.75	0.66–5.52	0.265 ¹	0.57	0.36–0.91	0.413 ¹
Female	1.40	0.39–5.98		0.53	0.35–0.83	
Stage						
I	1.07	0.41–5.43	0.336 ²	0.55	0.39–0.98	0.267 ²
II	1.58	0.40–3.38		0.47	0.31–0.78	
III	1.62	0.65–5.83		0.55	0.35–0.79	
IV	2.81	0.51–10.0		0.59	0.38–1.11	
Tumor classification (pT)						
pT1–2	1.12	0.41–4.32	0.047¹	0.54	0.36–0.81	0.838 ¹
pT3–4	1.84	0.61–7.77		0.55	0.35–0.88	
Lymph node metastasis (pN)						
pN0	1.33	0.41–4.54	0.391 ¹	0.54	0.37–0.88	0.494 ¹
pN1–3	1.73	0.56–6.24		0.55	0.34–0.79	
Distant metastasis (pM)						
pM0	1.48	0.54–4.90	0.122 ¹	0.54	0.35–0.81	0.178 ¹
pM1	2.81	0.51–10.0		0.59	0.38–1.11	
Laurén classification						
Intestinal	2.75	0.88–7.65	0.006¹	0.48	0.33–0.71	0.004¹
Diffuse	1.15	0.41–5.30		0.61	0.39–0.98	

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor,

CRP = C-reactive protein, IQR = interquartile range

¹Mann-Whitney U-test, ²Kruskal-Wallis test

Supplementary Table 4. Univariable survival analyses with median cut-off values for TAT-2 and TATI. Median TATI was 15.94 ng/ml and median TAT-2 was 8.68 ng/ml.

	HR	95% CI
Serum TAT-2 (ng/ml)		
<8.68	1.00	
≥8.68	1.46	1.04–2.04
Serum TATI (ng/ml)		
<15.94	1.00	
≥15.94	1.61	1.15–2.25

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, HR = hazard ratio, CI = confidence interval

Table 1. Association of serum TAT-2, TATI, and TAT-2/TATI ratio with clinicopathologic variables and CRP among 240 gastric cancer patients.

	Serum TAT-2 (ng/ml)			Serum TATI (ng/ml)		
	Median	IQR	P value	Median	IQR	P value
Age, years						
<67	8.95	6.59–12.9	0.174 ¹	14.0	11.0–20.7	0.001¹
≥67	8.10	5.17–14.05		18.2	12.1–27.4	
Gender						
Male	9.21	5.85–15.3	0.171 ¹	16.1	11.3–24.6	0.487 ¹
Female	8.11	5.91–11.3		15.7	10.8–22.2	
Stage						
I	8.39	5.25–11.8	0.585 ²	13.4	10.5–20.1	0.284 ²
II	8.89	6.39–13.1		18.0	11.6–26.5	
III	8.56	5.46–12.9		15.7	11.2–23.9	
IV	9.10	6.60–18.5		16.0	11.3–23.4	
Tumor classification (pT)						
pT1–2	7.76	5.00–11.1	0.046¹	13.8	10.6–20.0	0.030¹
pT3–4	9.07	6.30–14.3		16.3	11.6–24.6	
Lymph node metastasis (pN)						
pN0	8.79	6.31–12.4	0.602 ¹	16.0	10.6–23.5	0.479 ¹
pN1–3	8.56	5.33–13.3		16.0	11.3–23.6	
Distant metastasis (pM)						
pM0	8.59	5.64–12.7	0.175 ¹	15.9	11.2–23.6	0.893 ¹
pM1	9.10	6.60–18.5		16.0	11.3–23.4	
Laurén classification						
Intestinal	7.89	5.30–12.7	0.088 ¹	17.7	11.7–26.5	0.064 ¹
Diffuse	9.17	6.33–14.0		15.3	10.8–22.0	
CRP						
<10	8.76	5.88–13.1	0.523 ¹	15.7	11.1–22.4	0.037¹
≥10	8.59	6.40–18.3		19.2	13.0–30.0	
Serum TAT-2						
Low				14.2	10.6–18.6	<0.001¹
High				24.6	17.7–34.3	
Serum TATI						
Low	7.47	4.97–10.3	<0.001¹			
High	13.2	8.10–21.3				

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reacti

Table 2. Uni- and multivariate survival analyses for 240 gastric cancer patients.

	Univariate survival analysis			Multivariate survival analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age, years						
<67	1.00			1.00		
≥67	1.44	1.07–1.94	0.015	2.32	1.61–3.33	<0.001
Stage						
I	1.00			1.00		
II	4.97	2.05–12.0	<0.001	6.10	2.08–17.8	0.001
III	14.8	6.46–34.0	<0.001	23.0	8.32–63.5	<0.001
IV	41.7	17.7–98.1	<0.001	87.8	30.1–256	<0.001
Laurén classification						
Intestinal	1.00			1.00		
Diffuse	1.45	1.06–1.98	0.020	2.50	1.67–3.74	<0.001
CRP						
<10	1.00					
≥10	1.29	0.83–1.98	0.256			
Serum TAT-2						
Low	1.00			1.00		
High	1.93	1.35–2.76	<0.001	1.27	0.83–1.95	0.274
Serum TATI						
Low	1.00			1.00		
High	1.84	1.31–2.57	<0.001	2.01	1.32–3.07	0.001
Serum TAT-2/TATI ratio						
Low	1.00					
High	1.27	0.91–1.77	0.161			

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, HR = hazard ratio, CI = confidence interval

Table 3. Survival analyses by subgroups, high serum TAT-2, TATI, and TAT-2/TATI ratio compared to low among 240 gastric cancer patients.

	High Serum TAT-2			High Serum TATI		
	HR	95% CI	P value	HR	95% CI	P value
Age, years						
<67	1.66	0.99–2.76	0.053	1.63	1.00–2.68	0.052
≥67	2.24	1.35–3.70	0.002	1.86	1.15–3.02	0.012
Gender						
Male	1.87	1.13–3.11	0.015	1.80	1.09–2.97	0.023
Female	2.12	1.27–3.55	0.004	1.87	1.19–2.96	0.007
Stage						
I	4.73	0.66–33.6	0.121	3.48	0.48–25.1	0.217
II	2.24	0.90–5.59	0.083	1.68	0.71–3.97	0.235
III	1.42	0.85–2.38	0.186	2.13	1.33–3.41	0.002
IV	2.25	1.15–4.41	0.018	2.37	1.12–4.62	0.012
Tumor classification (pT)						
pT1–2	0.97	0.22–4.35	0.971	1.42	0.44–4.54	0.557
pT3–4	1.70	1.17–2.47	0.005	1.57	1.10–2.23	0.013
Lymph node metastasis (pN)						
pN0	3.28	1.27–8.50	0.014	3.56	1.38–9.23	0.009
pN1–3	1.61	1.08–2.42	0.021	1.81	1.24–2.65	0.002
Distant metastasis (pM)						
pM0	1.73	1.12–2.67	0.014	1.94	1.31–2.89	0.001
pM1	2.25	1.15–4.41	0.018	2.37	1.21–4.62	0.012
Laurén classification						
Intestinal	2.19	1.05–4.57	0.037	2.10	1.10–4.02	0.025
Diffuse	1.76	1.17–2.66	0.007	1.86	1.25–2.78	0.002
CRP						
<10	1.65	1.10–2.47	0.015	1.67	1.14–2.44	0.008
≥10	3.67	1.64–8.22	0.002	2.51	1.08–5.84	0.033
Serum TAT-2						
Low				1.80	1.15–2.83	0.010
High				1.02	0.52–2.02	0.945
Serum TATI						
Low	2.30	1.21–4.36	0.011			
High	1.25	0.75–2.08	0.386			

Abbreviations: TAT-2 = tumor associated trypsin-2, TATI = tumor associated trypsin inhibitor, CRP = C-reactive protein, HR = hazard ratio, CI = confidence interval

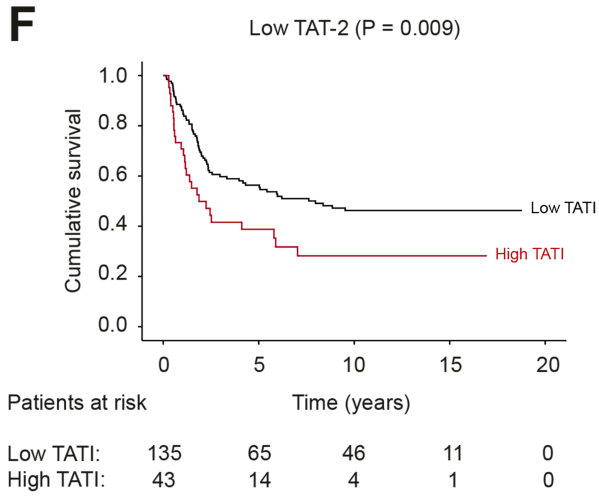
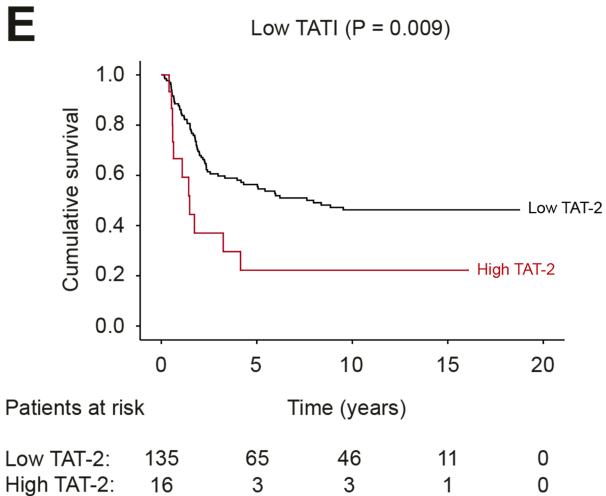
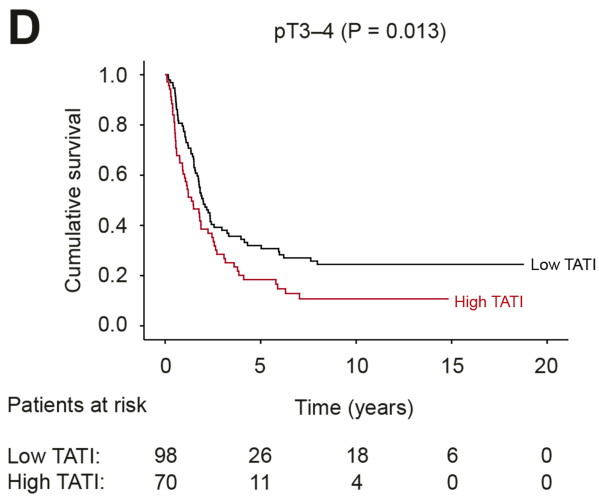
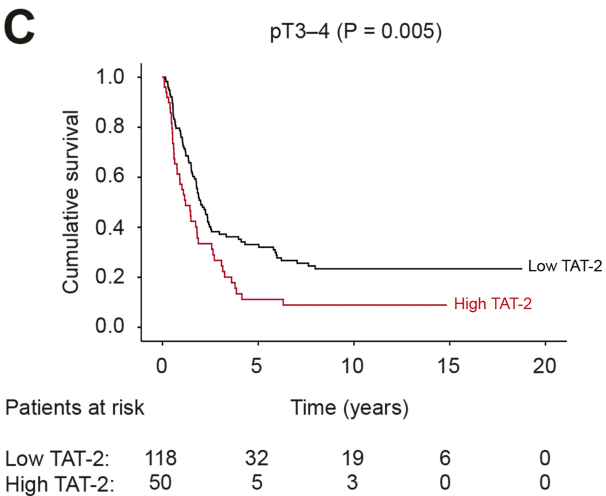
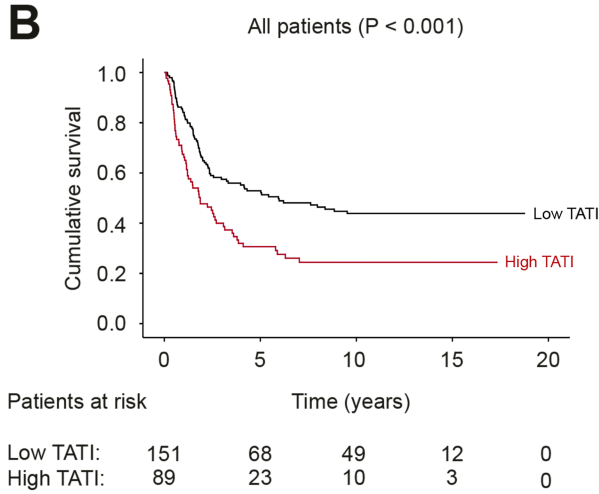
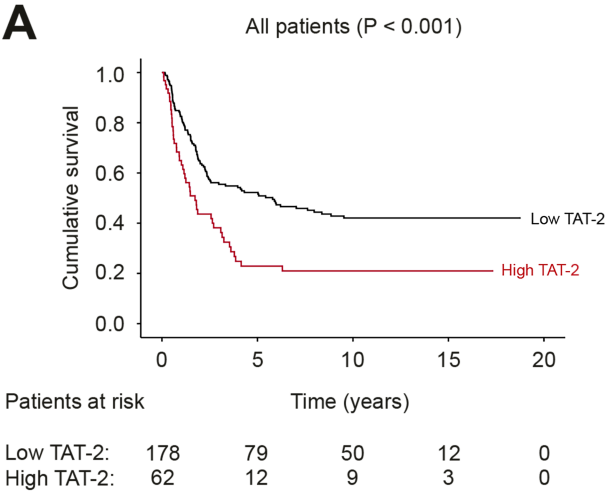


Figure 1

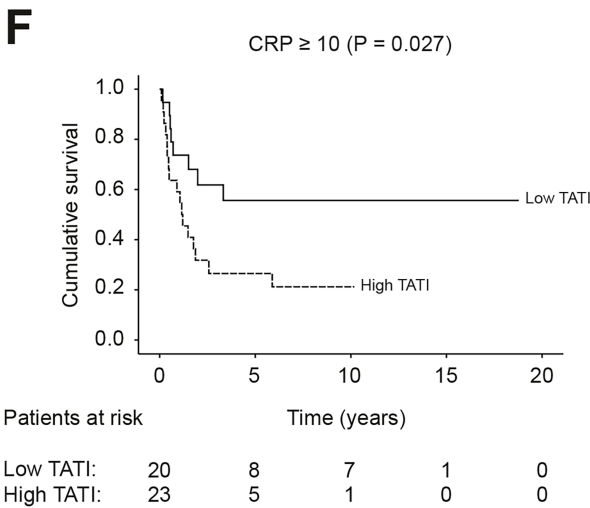
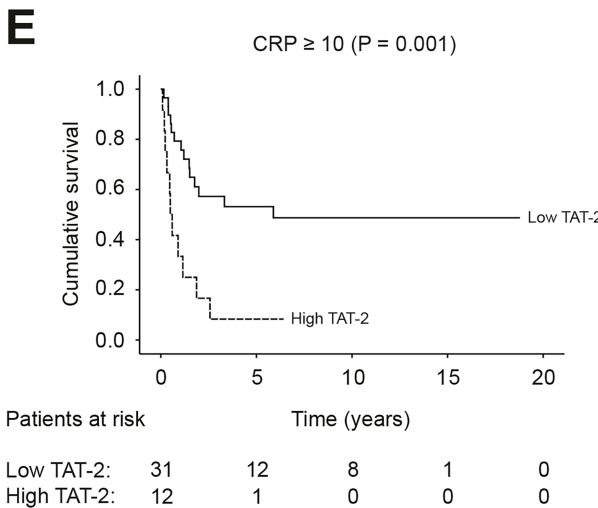
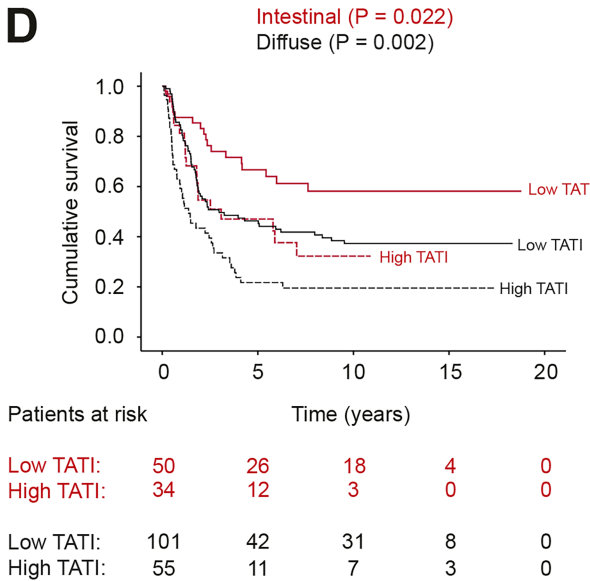
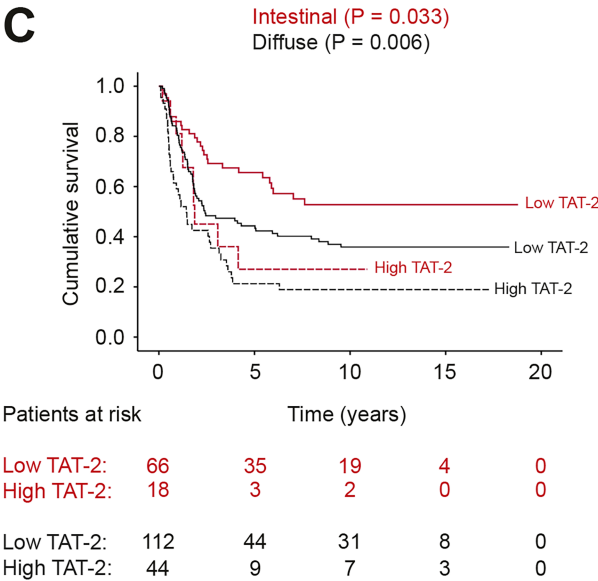
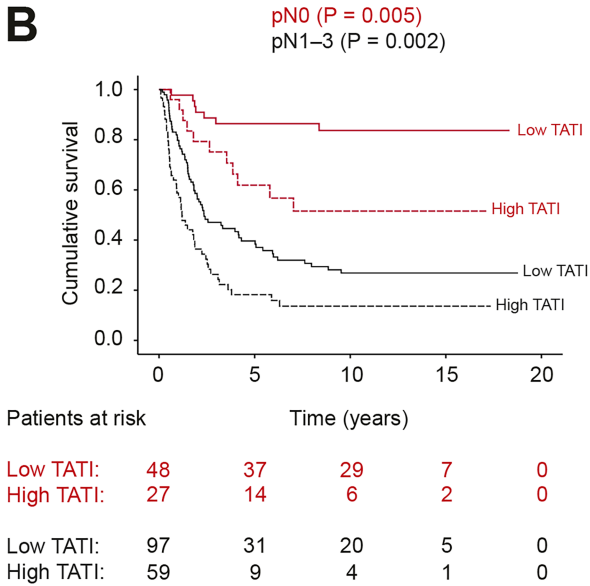
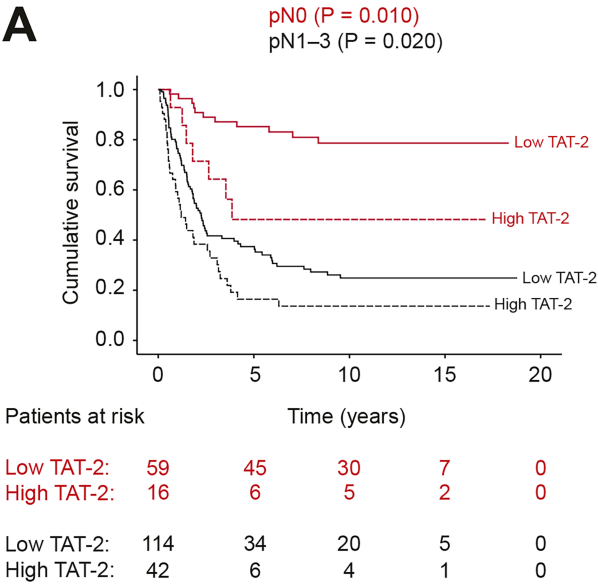


Figure 2